Plant Gene Register 00-012 *Plant Physiol.* (2000)122:291

Isolation of a cDNA Encoding a Rac-like GTP-Binding Protein (Accession No. AF165925) from Cotton (Gossypium hirsutum L.).

Hee Jin Kim, John Charalambopoulos and Barbara A. Triplett*

USDA, ARS, Southern Regional Research Center, New Orleans, Louisiana 70179 (HJK, BAT), Howard Hughes Medical Foundation Intern Program, Department of Biology, University of New Orleans, New Orleans, Louisiana 70148 (JC)

*Corresponding Author; email: btriplet@nola.srrc.usda.gov; FAX: 1-504-286-4419

GTP-binding proteins (G-proteins) are important regulators of signaling pathways in eukaryotic organisms. Small G-proteins in plants are involved in diverse functions and have been classified into distinct families. The Rab family of G-proteins regulates the specificity and directionality of intracellular protein traffic. We have recently characterized two members of the Rab family from developing cotton fiber (Charalambopoulos et al., 1999). The Ran family plays a key role in the nuclear protein import system. Arf and Sar families are important regulators of membrane traffic and organelle structure. The Rho family of yeast and mammals is linked to the regulation of a wide range of processes, including the organization of the actin cytoskeleton. Plant Rho-like G-proteins are divided into two groups: either Rop or Rac-like G-proteins. Rac-like G-proteins are 50 % identical to Rop proteins, however, the Rac-like G-proteins have a higher molecular weight and a C-terminal motif for prenylation. Post-translational prenylation of Rac-like G-proteins firmly anchors the protein to the cell membrane through complex glycosyl-phosphatidylinositols attached to the protein's terminal carboxyl group (Low and Saltiel, 1988). This type of modification is crucial for biological activities of ras proteins.

In this study, one cDNA (GhRac1) encoding a small G-protein was obtained from developing cotton locules using RT and 5' RACE PCR. Previously, two other cotton G-protein cDNAs were characterized (S79309 and S79308) and are members of the Rop family although they were initially named "Rac 9" and "Rac 13". While GhRac1 is highly homologous (78 %) to "Rac 9" and "Rac 13", the presence of a C-terminal farnesylation motif suggests that GhRac1 is more appropriately grouped with the Rac-like G-proteins. Motif searching with ProSite shows that GhRac1 has a C-terminal prenyl group binding motif (position 195-198). A BLASTP homology search shows that GhRac1 is equally homologous (80 %) to Rop G-proteins (U62746, U49971, L19093) and other Rac-like G-proteins (U52350, Z73961).

In mammalian phagocytes, Rac1 protein not only activates a NADPH-dependent oxidase resulting in accumulation of hydrogen peroxide, but also controls actin redistribution to membrane ruffles in fibroblasts (Moldovan et al., 1999). The Rho family of G-proteins is involved in control of cell morphology and also mediates signals from cell membrane receptors (Winge et al., 1997). Members of the Rop family in cotton, "Rac 13" and "Rac 9" showed highly induced expression at the transition from primary to secondary wall synthesis (Delmer et al., 1995). Constitutive expression of the cotton "Rac13" gene induced the production

of hydrogen peroxide in cultured soybean and Arabidopsis cells. Thus, it has been suggested that "Rac 13" in cotton plays a role in the activation of NADPH oxidase and generation of hydrogen peroxide (Potikha et al., 1999). Another homologous G-protein, Rop1Ps is found in pollen tubes of pea. Microinjected anti-Rop1Ps antibodies inhibited pollen tube elongation, suggesting that it might be involved in controlling actin-dependent tip growth and possibly actin-dependent movement of the generative cell (Lin and Yang, 1997).

As in other Rac-like G-proteins in plants or Rac1 in phagocytes or fibroblasts, it is likely that GhRac1 is localized on the plasmamembrane in cotton cells. Assigning an exact role that GhRac1 plays in cotton fiber and/or ovule development, however, must await additional experiments.

ACKNOWLEDGMENTS

This project was funded by the Howard Hughes Medical Foundation grant to the University of New Orleans, Department of Biological Sciences and by the USDA-ARS CRIS 6435-21440-001-00D

LITERATURE CITED

Bischoff F, Molendijk A, Rajendrakumar CSV, Palme K (1999) GTP-binding Proteins in Plants. Cell Mol Life Sci. 55: 233-256

Borg S, Brandstrup B, Jensen TJ, Poulsen C (1997) Identification of new protein species among 33 different small GTP-binding proteins encoded by cDNAs from Lotus japonicus, and expression of corresponding mRNAs in developing root nodules. The Plant J 11: 237-250

Charalambopoulos J, Kim HJ, Triplett BA (1999) Characterization of two cDNAs encoding small GTP-binding proteins (Accession Nos. AF165095 and AF165096) from immature cotton (_Gossypium hirsutum_ L.) locules. Plant Gene Register, in press.

Delmer DP, Pear JR, Andrawis A, Stalker DM (1995) Genes encoding small GTP-binding proteins analogous to mammalian Rac are preferentially expressed in developing cotton fiber. Mol Gen Genet 248: 43-51

Lin Y, Yang Z (1997) Inhibition of pollen tube elongation by microinjected anti-Rop1Ps antibodies suggests a crucial role for Rho-type GTPase in the control of tip growth. Plant Cell 9: 1647-1659

Low MG, Saltiel AR (1988) Structural and functional roles of glycosyl-phophatidylinositol in membranes. Science 239: 268-275

Moldovan L, Irani K, Moldovan NI, Finkel T, Goldschmidt-Clermont PJ (1999) The actin cytoskeleton reorganization induced by rac1 requires the production of superoxide. Antioxidants & Redox Signaling 1: 29-43

Potikha TS, Collins CC, Johnson DI, Delmer DP, Levine A (1999) The involvement of hydrogen peroxide in the differentiation of secondary walls in cotton fibers. Plant Physiol. 119: 849-858

Winge P, Brembu T, Bones AM (1997) Cloning and characterization of rac-like cDNAs from Arabidopsis thaliana. Plant Mol Biol 35: 483-495

TABLE I. Characterization of GhRac1 from Immature Cotton Locules

Organism:
Upland cotton, *Gossypium hirsutum* L., Texas Marker-1

Source:
PolyA+ RNA from 6 days post anthesis (DPA) cotton locules was reverse transcribed and PCR amplified using primers that were designed from conserved sequences in the Rac family of small G-proteins. Full-length cDNA clones were obtained using 5' Rapid Amplification of cDNA Ends (5' RACE). Amplified products were cloned in the plasmid PCRII (Invitrogen).

Sequencing:
Both strands of each plasmid were sequenced using the 4000 L Automated DNA Sequencer (LI-COR) with the cycle sequencing protocol from the SequiTherm EXCEL II Long-Read Sequencing Kit-LC (Epicentre Technologies).

Features of the cDNA:
The cDNA encoding GhRac1 is 951 bp in length and contains a 5' UTR (108 bp) and a 3' UTR (246 bp).

Features of the predicted amino acid sequence:
The open reading frame of GhRac1 consists of 198 amino acids. A motif search using ScanProsite revealed a GTP-binding motif (position 13-20), an N-glycosylation site (position 42-45), two N-myristoylation sites (positions 51-56 and 57-62), and several phosphorylation sites of cAMP-dependent protein kinase (position 64-67), protein kinase C (positions 69-71 and 159-161), CK2 (positions 11-14 and 38-41) and tyrosine kinase (positions 99-107). The C-terminal consensus sequence for farnesylation found on many Rac-like G-proteins is present in GhRac1 (position 195-198).

Footnote: Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.